

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date
3 February 2005 (03.02.2005)

PCT

(10) International Publication Number
WO 2005/010145 A3

- (51) International Patent Classification⁷: **C07H 21/04, C12Q 1/68**
- (21) International Application Number: **PCT/US2004/015587**
- (22) International Filing Date: **9 June 2004 (09.06.2004)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
60/485,301 5 July 2003 (05.07.2003) US
60/525,859 1 December 2003 (01.12.2003) US
- (71) Applicant (*for all designated States except US*): **THE JOHNS HOPKINS UNIVERSITY [US/US]; 3400 N. Charles Street, Baltimore, MD 21218 (US).**
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **DRESSMAN, Devin [US/US]; 701 S. Luzerne Avenue, Baltimore, MD 21224 (US). YAN, Hai [CN/US]; 7236 Montgomery Road #3C, Elkrige, MD 21075 (US). KINZLER, Kenneth, W. [US/US]; 1403 Halkirk Way, Baltimore, MD 21015 (US). VOGELSTEIN, Bert [US/US]; 3700 Breton Way, Baltimore, MD 21208 (US).**
- (74) Agent: **KAGAN, Sarah, A.; Banner & Witcoff, Ltd., 11th Floor, 1001 G Street, N.W., Washington, DC 20001-4597 (US).**
- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

(88) Date of publication of the international search report:
11 August 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/010145 A3

(54) Title: **METHOD AND COMPOSITIONS FOR DETECTION AND ENUMERATION OF GENETIC VARIATIONS**

(57) **Abstract:** Many areas of biomedical research depend on the analysis of uncommon variations in individual genes or transcripts. Here we describe a method that can quantify such variation at a scale and ease heretofore unattainable. Each DNA molecule in a collection of such molecules is converted into a single particle to which thousands of copies of DNA identical in sequence to the original are bound. This population of beads then corresponds to a one-to-one representation of the starting DNA molecules. Variation within the original population of DNA molecules can then be simply assessed by counting fluorescently-labeled particles via flow cytometry. Millions of individual DNA molecules can be assessed in this fashion with standard laboratory equipment. Moreover, specific variants can be isolated by flow sorting and employed for further experimentation. This approach can be used for the identification and quantification of rare mutations as well as to study variations in gene sequences or transcripts in specific populations or tissues.